Immigration delays but does not prevent adaptation following environmental change: experimental evidence

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Abstract

In today's rapidly changing world, it is critical to thoroughly examine how animal populations will respond to severe environmental change. Following events such as pollution or deforestation that cause populations to decline, extinction will occur unless populations can adapt in response to natural selection, a process called evolutionary rescue. Theory predicts that immigration can delay extinction and provide novel genetic material that can prevent inbreeding depression and facilitate adaptation. However, when potential source populations have not experienced the new environment before (*i.e.,* are naive), immigration can counteract selection and constrain adaptation. This study evaluated the effects of immigration of naive individuals on evolutionary rescue using the red flour beetle, *Tribolium castaneum,* as a model system. Small populations were exposed to a challenging environment, and three immigration rates (zero, one, or five migrants per generation) were implemented with migrants from a benign environment. Following an initial decline in population size across all treatments, populations receiving no immigration gained a higher growth rate one generation earlier than those with immigration, illustrating the constraining effects of immigration on adaptation. After seven generations, a reciprocal transplant experiment found evidence for adaptation regardless of immigration rate. Thus, while the immigration of naive individuals briefly delayed adaptation, it did not increase extinction risk or prevent adaptation following environmental change.

Keywords: evolutionary rescue, dispersal, *Tribolium castaneum*, gene flow, translocation

41 # 1 | Introduction

42 A critical goal of conservation biology is to establish how to effectively manage
43 populations in human-altered habitats. Environmental change such as pollution (Loria *et al.*,
44 2019), deforestation (Foster *et al.*, 2021), and fragmentation (Cheptou *et al.*, 2017) can reduce
45 habitat quality and connectivity, which can decrease the average fitness of residing
46 populations. If fitness drops below the replacement rate, populations will decline in size and
47 will approach extinction if the downward trend persists. Immigration, either through
48 maintenance of dispersal corridors or translocations, is one approach readily available to
49 conservation practitioners to support declining populations, particularly in fragmented
50 landscapes (Frankham *et al.*, 2019).

51 Theoretical work within the conservation literature recommends an immigration rate of
52 one to ten migrants per generation for isolated populations (Mills & Allendorf, 1996; Frankham
53 *et al.*, 2019). More specifically, theory suggests a rate of one migrant per generation to offset
54 population divergence and loss of heterozygosity that occurs through drift (Mills & Allendorf,
55 1996), and five migrants per generation to prevent damaging inbreeding depression and
56 maintain adaptive genetic variation in changing environments (Frankham *et al.*, 2019). Ideally,
57 immigration can occur consistently through dispersal corridors, and if translocations are
58 needed, consistent immigration should be sustained. However, these recommendations have
59 been tested only partially. Laboratory experiments have demonstrated the benefits of
60 immigration when comparing isolated populations to populations that received a single
61 immigration event under scenarios of inbreeding (Spielman & Frankham, 1992) or low diversity
62 populations facing environmental change (Hufbauer *et al.*, 2015). Only one study we know of,

Newman & Tallmon (2001), tested immigration that occurred every generation. They assessed rates of one or 2.5 migrants per generation in recently isolated mustard plant populations, and found that the rate of one migrant per generation was sufficient to maintain genetic variation and decrease inbreeding when compared to isolated populations (Newman & Tallmon, 2001). In this era of global change, it is important to also test these recommendations under more severe environmental conditions.

A separate but related body of literature focuses on how populations declining due to exposure to challenging environments can avoid extinction through adaptive evolution, a process called evolutionary rescue (Gomulkiewicz & Holt, 1995; Carlson *et al.*, 2014). Examples include adaptation in weed or insect populations to pesticides, antibiotic resistance in bacteria, or invertebrate adaptation to metal pollution in streams (Bell, 2017). Experiments show that immigration or admixture can enhance the probability of evolutionary rescue if populations are exposed to a challenging environment (Hufbauer *et al.*, 2015; Durkee *et al.*, 2023). Furthermore, theoretical studies show that the probability of rescue can increase with immigration, primarily due to the added genetic variation, which increases the chance of beneficial alleles being present (Tomasini & Peischl, 2020; Czuppon *et al.*, 2021). To contrast, other theoretical studies show that gene flow from maladapted immigrants can limit adaptation by swamping adaptive alleles in the recipient population, reducing fitness (*i.e.,* outbreeding depression) (Slatkin, 1987; Lenormand, 2002; Brady *et al.*, 2019).

To reduce the likelihood of outbreeding depression, using a source population from a habitat similar to the recipient population (*i.e.,* habitat matching) is recommended by models (Edelaar & Bolnick, 2012) and in the context of conservation (Frankham *et al.*, 2019). However,

85 this is often not possible, particularly under scenarios of novel environmental change (Aitken &
86 Whitlock, 2013) or when the species of concern is rare (Frankham *et al.*, 2019). Even when
87 migrants are not adapted to their new habitat, for example when individuals from a
88 geographically distant population (*e.g.,* Hedrick & Fredrickson, 2010) or from captivity (*e.g.,*
89 Crone *et al.,* 2007) are used, every-generation immigration of one to five individuals is meant to
90 be low enough to minimize outbreeding depression while still maintaining genetic diversity and
91 adaptive potential of populations (Newman & Tallmon, 2001; Frankham *et al.*, 2019). However,
92 to our knowledge, no study has compared the recommended immigration rates of one and five
93 migrants per generation and their impacts on adaptation in the context of severe
94 environmental change.

95 Here, we evaluated how immigration rates recommended in the conservation literature
96 (Frankham *et al.*, 2019) impacted evolutionary rescue (Carlson *et al.*, 2014). To do this, we first
97 created experimental populations of red flour beetles (*Tribolium castaneum*) from a genetically
98 admixed stock population (Durkee *et al.,* 2023). We then introduced them to a challenging
99 environment to mimic a situation where a large, diverse population experiences sudden habitat
100 change that decreases population size and reduces habitat quality, for example when forest are
101 logged (Hillers *et al.,* 2008). Each experimental population received one of three immigration
102 treatments (zero, one, or five individuals every generation), and we observed the effects of
103 immigration on population persistence and growth. In habitats like these that have limited
104 space and degraded resources, population recovery may be constrained by density-dependent
105 processes like competition for food and space (Osmond & de Mazancourt, 2013; Nordstrom *et*
106 *al.*, 2023). Thus, we evaluated both density-dependent and density-independent population

growth through time to tease apart the impacts of negative density dependence and adaptation. Our experiment was designed to address the following question: In populations experiencing severe environmental change, how do different immigration rates affect *(i)* population size and growth rates over time, *(ii)* the timing of evolutionary rescue, and *(iii)* adaptation to the challenging environment?

2 | Methods

To evaluate the question posed in the introduction, we carried out an experiment where we subjected replicated, independent populations to a challenging environment that caused them to decline in size. This environment, therefore, would have led to extinction unless the populations were able to adapt sufficiently to the challenging conditions. Hereafter, we refer to this as the rescue experiment (Figure 1a-b).

Rescue experiment initiation and propagation

We created a genetically diverse *Tribolium castaneum* stock population comprised of lineages described in Durkee *et al.* (2023). The stock population had been reared on standard media (19:1 wheat flour to brewer's yeast) and in standard environmental conditions (31°C and 50-80% humidity) in 4x4x6cm patches for nine, 35-day non-overlapping generations prior to the start of the rescue experiment. To start, we created 25 independent populations from the stock population of 300 individuals each (Figure 1a). These populations were used to initiate replicated experimental populations and maintain populations to serve as a source of migrants

129 (hereafter, source populations). These populations were divided at the start of the rescue
130 experiment, and therefore represent newly isolated populations with no history of divergence,
131 similar to Newman & Tallmon (2001).

132 **Experimental populations** were each initiated with 50 adult beetles, 36 days old, and
133 population sizes were allowed to fluctuate naturally in subsequent generations. Each
134 population was placed onto a single patch of challenging environment created using media
135 containing the pyrethroid insecticide deltamethrin (DeltaDust, Bayer). This event represents a
136 sudden environmental change that reduced fitness and created replicated, isolated
137 populations. The experimental patches each contained a concentration of deltamethrin that a
138 dose-response curve created from preliminary experiments (Figure S1) indicated would
139 decrease the population growth rate to approximately 0.40 from a typical value of around 2.
140 Without adaptation, this reduced growth rate should have led to extinction in 10 generations
141 (based on simulations with the stochastic Ricker model developed for *T. castaneum* by
142 Melbourne & Hastings, 2008). The experimental populations were initiated in 25 groups of
143 three, each group from one of the original 25 independent populations (Figure 1a).

144 **Source populations** were initiated with 150 adults, 36 days old, and each served as a
145 source of migrants for a group of three experimental populations (Figure 1a). We intended for
146 the sources to represent larger populations, and so we used the large population size (N=150)
147 from Hufbauer *et al.* (2015). Sources were maintained at 150 adults every generation regardless
148 of the number of offspring produced, to approximate populations with a carrying capacity of
149 150 individuals. Source populations were each maintained on a single patch containing
150 standard dry media, a benign environment for *T. castaneum*. Thus, these individuals were not

exposed to the challenging environment present in the experimental patches. Hereafter, we refer to these individuals as naive migrants, to refer to both evolutionary and behavioral naivety.

The adult beetles in the experimental and source patches were given 24 hours to mate and oviposit. Then they were discarded, and the eggs developed for an additional 34 days. The first census event (N_1, Figure 1b) occurred on day 35. Subsequently, one of three immigration treatments was applied to each experimental population in every group: zero, one, or five migrants. The migrants were moved from the source to each experimental population manually using a paintbrush. This occurred at the start of the following oviposition period. This procedure (immigration of one or five migrants, 24-hour oviposition, removal of adults, 34-day development period, census) repeated each subsequent generation. The experiment was terminated after seven generations because nearly all the experimental populations had either gone extinct or their growth rates and population sizes had increased, indicative of evolutionary rescue (see Figure 2).

In total, there were 25 groups, each consisting of one source and three experimental populations (one of each treatment). Thus, there were 25 replicates of each treatment, split across three temporal blocks (eight groups in blocks 1 and 2, nine in block 3; Figure 1a). Within each group, treatments were set up sequentially, and propagated for the first four generations in the following order to minimize opportunities for experimental error: 1-migrant, 5-migrants, and then the 0-migrant populations. As this was not random, we tested if this order mattered in a follow-up experiment and found no significant effect of treatment order on the growth rate

172 the following generation (Figure S2). During generations 4 through 7, the experimental protocol
173 was well established, and so we randomized the order of set-up.
174
175 *Source population dispersal arrays*
176 The immigration recommendations we followed (one or five migrants per generation)
177 are based on either natural dispersal or translocation. Thus, in order to approximate natural
178 dispersal and make our results applicable to either case, we used migrants with higher
179 demonstrated dispersal ability to represent individuals that would be more likely to leave the
180 source patch and move to a new patch in the absence of translocation (Edelaar & Bolnick, 2012;
181 Cote *et al.*, 2022). Dispersal arrays were created to select dispersing individuals. On day 34
182 during generations 1-6, habitat patches housing source populations were connected to 3-patch
183 dispersal arrays connected with 2mm holes, creating a 4-patch dispersal array. Hereafter, we
184 refer to the original source patch as patch 1 and the subsequent patches, in order, as patches 2-
185 4, which contained media that had been previously used to rear beetles for 35 days to keep
186 resource availability consistent throughout the array. Adult beetles were given 24 hours to
187 disperse out of patch 1. On day 35, all adults were censused in each of the four patches of the
188 dispersal array (Figure S3). Individuals that dispersed to patches 2-4 were used as migrants
189 (hereafter called dispersing migrants) with preference given to individuals that dispersed to the
190 furthest patch (*i.e.,* individuals were first chosen from patch 4, then patch 3, then 2).
191 In order to maintain the population size of the source populations at 150 to start every
192 generation, growth rates needed to be above the replacement rate. However, growth rates
193 were sometimes lower than expected, which likely occurred due to effects of demographic

194 stochasticity, which strongly influences the *T. castaneum* system (Melbourne & Hastings, 2008).
195 When this happened, populations were supplemented with individuals from the stock
196 population at the start of the oviposition period, after the dispersal period and immigration to
197 the experimental populations occurred. After the first generation, two source populations were
198 initialized with 110 individuals due to limited availability of stock individuals.
199
200 *Common environment and reciprocal transplant experiment*
201 After seven generations, we terminated the rescue experiment, and all extant
202 populations (58 experimental and 25 source populations) were placed at a constant density of
203 40 individuals on standard media for a single generation (hereafter, "common environment"
204 generation; Figure 1c). All adults were kept within their experimental populations, *i.e.,* no
205 mixing occurred within or across treatments. In *T. castaneum,* maternal environmental effects
206 can strongly affect growth rate (Hufbauer *et al.,* 2015; Van Allen & Rudolf, 2013), and so this
207 generation where the environment and density were kept the same for all populations served
208 to standardize maternal effects for the subsequent reciprocal transplant experiment. With only
209 a single generation, we do not standardize grand-maternal effects. However, inference from
210 multigenerational studies suggests that grand maternal effects are relatively weak in *T.*
211 *castaneum* populations (Van Allen & Rudolf, 2013; Hufbauer *et al.,* 2015).
212 In the ninth generation, a reciprocal transplant experiment was completed to assess
213 adaptation to the challenging environment and potential costs to adaptation in the source
214 environment (Figure 1d). We divided individuals from each population into three groups and
215 randomly assigned them to one of three environments: standard media (0ppm, hereafter

216 “source environment”), media containing the concentration of deltamethrin present in the
217 challenging environment of the rescue experiment (2.968ppm, hereafter “experimental
218 environment”), and media containing a higher concentration of deltamethrin (5.641ppm,
219 hereafter “severe environment”). The dose-response curve (Figure S1) indicated that the severe
220 environment would reduce the growth rate of naive populations to near zero. Each replicate
221 patch of these three environmental treatments received 40 beetles, which were allowed 24
222 hours for oviposition, and 34 additional days for development prior to census. Replication
223 within population depended on the number of available individuals following the common
224 environment generation (see Figure S4).
225

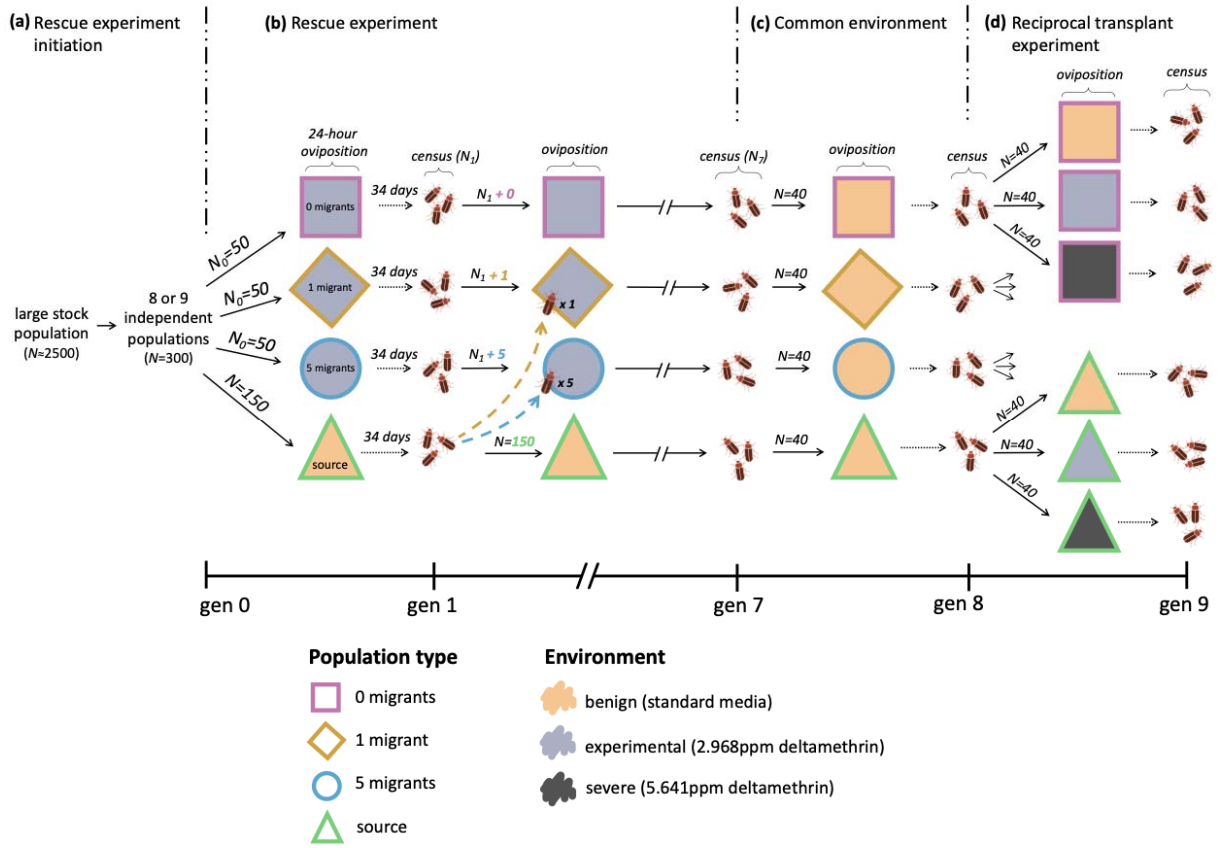

Figure 1. Experimental design for the rescue and reciprocal transplant experiments. a) We began with a large stock population (*N*≈2500), from which eight or nine separate populations were initiated (*N*=300). Each of these populations was split into one source population, initiated and then maintained at *N*=150 individuals, and three experimental populations, initiated with N_0=50 individuals with population size allowed to fluctuate thereafter. The source populations were maintained on a benign environment (tan shading), and the experimental populations were maintained in a challenging environment containing the insecticide deltamethrin (light gray shading). **b)** In the rescue experiment, census events (gens 1 & 7 shown) are indicated with three cartoon beetles, and census occurred every generation on day 35, following a 24-hour oviposition period and 34 days of development (indicated with dotted

arrows). Immigration from the source to the experimental populations (dashed arrows) occurred after each census event at the start of the oviposition period, and three treatments were implemented: 0 migrants (purple square outlines), 1 migrant (yellow diamond outlines), and 5 migrants per generation (blue circle outlines). **c)** All extant populations were placed on a benign, common environment for a single generation following the census in generation seven at a density of *N*=40, with as many replicates created as possible. **d)** In the reciprocal transplant experiment, all populations were split into groups of 40 and placed simultaneously and at constant densities on three different environments: benign, challenging, and severe (dark gray shading). This whole procedure **(a-d)** was repeated across three temporal blocks, with eight replicates in blocks 1 and 2 and nine replicates in block 3.

Data analysis

All statistical analyses were performed in R version 4.1.2 (R Core Team, 2021). Population size at census, which occurred on day 35 during generations 1-7, was used to assess population dynamics throughout the rescue experiment. Populations were considered rescued if they grew to a population size greater than 50, and maintained a growth rate greater than 1 (the replacement rate) for at least two of the final four generations. This allowed for decreases in population size due to negative density dependence. Extinction risk and survival times were quantified using a survival analysis with the *ggsurvfit* function in the *survival* package (Therneau 2021). Extinct populations were defined as those with zero adults at census during the first six

258 generations or one adult by generation seven. Overall extinction rates were calculated as raw
259 percentages with confidence intervals around each percentage for each treatment.

260 Population growth was calculated as the mean number of surviving adult offspring per
261 individual in the population, *i.e.,* population size at census each generation (N_t) divided by the
262 number of parents that produced those individuals (parent population size, N_{t-1}), or N_t / N_{t-1}.
263 We evaluated both density-dependent and density-independent growth rates through time
264 using a linear mixed model with the *lme4* package (Bates *et al.*, 2015). We included immigration
265 treatment (0 migrants, 1 migrant, or 5 migrants per generation), time (t) in generations (from 1
266 to 7 generations, categorical), and parent population size (N_{t-1}, continuous) as fixed effects, and
267 temporal block (block 1, block 2, block 3), source population (nested within block), and
268 population identity (nested within source population) as random effects. Growth rate data
269 were square root transformed to ensure that the equal variance assumption was satisfied.

270 From this model, we generated estimates of both density-dependent and density-
271 independent population growth rates using the *predict* function with different values for the
272 starting population size (N_{t-1}) each generation. To estimate density-dependent growth rates, we
273 allowed the values for N_{t-1} to vary for each generation and treatment combination, matching
274 the observed mean population sizes at the start of each generation. This estimate was used to
275 describe the population growth rate data and quantify the effects of immigration treatment in
276 a finite habitat by considering the constraints of competition for resources and cannibalism that
277 affected the populations in our experiment (Pointer *et al.*, 2021). To estimate density-
278 independent growth rates from the fitted model, we set N_{t-1} to zero for every generation and
279 treatment, which is equivalent to estimating the y-intercepts when plotting model-estimated

280 growth rate over N_{t-1} (Figure S5). These density-independent estimates allowed us to track
281 adaptation to the environment by revealing the change in intrinsic fitness of individuals in the
282 population unfettered by the effects of density. We expected the density-independent growth
283 rates to be higher than the density-dependent estimates due to the known negative density
284 dependence in *T. castaneum* (Melbourne & Hastings 2008). Confidence intervals for both
285 density-dependent and density-independent growth rate estimates were estimated by
286 parametric bootstrap (percentile method) using the *bootMer* function with 5000 simulations in
287 the *lme4* package (Bates *et al.*, 2015). Comparisons between treatments were made by
288 estimating confidence intervals by parametric bootstrap (percentile method) for the difference
289 in means between treatments within each generation using the *simpleboot* and *boot* packages
290 with 5000 simulations (Peng, 2019; Canty & Ripley, 2022).

291 The timing of evolutionary rescue was assessed in each treatment group using a
292 Generalized Additive Mixed (GAM) model (Wood, 2017) similar to Olazcuaga *et al.* (2023). Only
293 rescued populations were considered in this analysis. We modeled the population size of
294 rescued populations using a negative binomial distribution to account for overdispersion using
295 the package *mgcv* (Wood, 2004, 2011). To allow for the relationship between the population
296 size and time to be nonlinear, we modeled population size as a smooth function of generation
297 with the number of knots set to 4, which was chosen to maximize the adjusted R-squared value.
298 Explanatory variables also included immigration treatment and the interaction of treatment
299 and generation as fixed effects, allowing for a smooth function for each treatment. The full
300 model included population identity and temporal blocks as random effects. The best-fit model

was chosen by comparing to reduced models using the *compareML* function from the *itsadug* package (van Rij *et al.*, 2022).

We used the reciprocal transplant data to further evaluate adaptation to the experimental environment, as well as potential fitness costs of adaptation in a benign environment. Growth rate in the reciprocal transplant experiment was used as the response variable in a linear mixed effects model that included population type (source, 0-migrant, 1-migrant and 5-migrant), test environment (source, experimental, severe), and the interaction between population type and test environment as fixed effects. Population identity was included as a random effect nested within temporal block. Comparisons within each environment and among immigration treatments were made using 95% bootstrapped confidence intervals (percentile method) for the difference in means, which were generated from 5000 iterations.

3 | Results

Population size and extinction

During the rescue experiment, a majority of populations experienced evolutionary rescue (Figure 2, black lines), and extinction probability was similar for all three treatments (Figure 2, red lines). The difference in the extinction risk was statistically similar across treatments throughout the experiment (Figure S6). Additionally, there was no clear difference in survival time among treatments ($\chi^2_2 = 0.1$, p = 1.00).

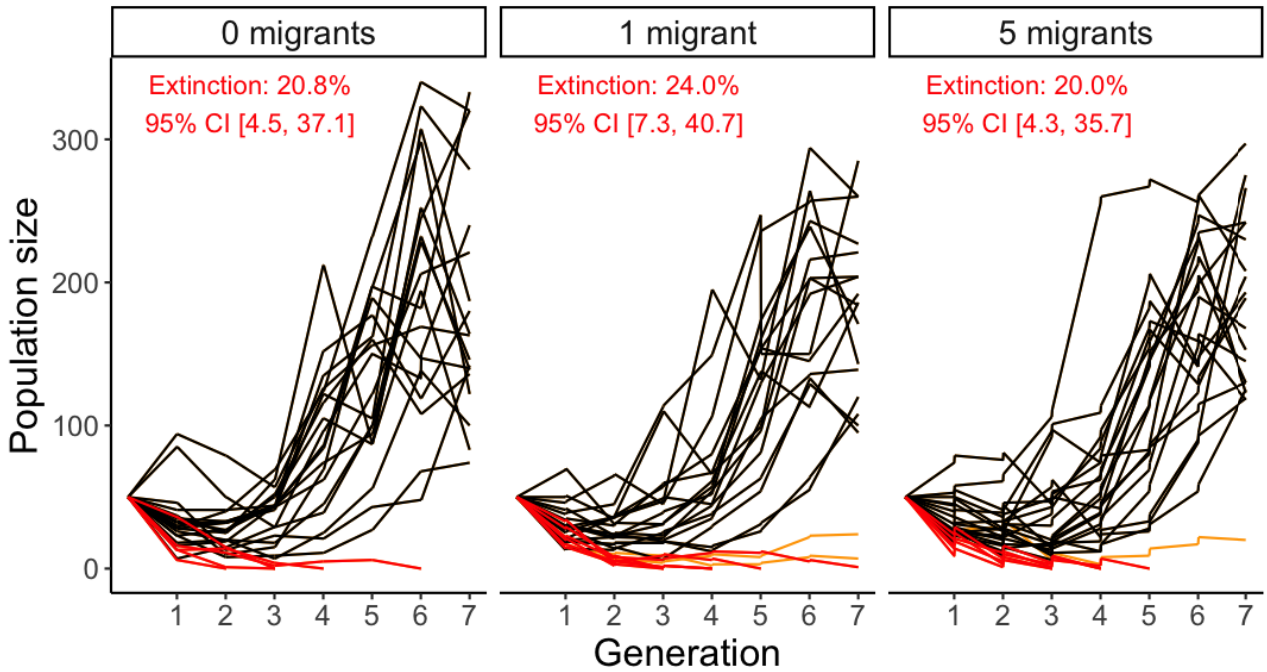

Figure 2. Population size through time in discrete generations for the populations receiving 0, 1, or 5 migrants per generation during the rescue experiment. Population sizes represent the number of adult beetles present at census (day 35 each generation). Each black line represents a rescued population, each orange line represents an extant population that was not considered rescued, and each red line represents a population that went extinct or had a population size of one individual in generation seven. Immigration events are indicated with instantaneous increases in population size, which occurred at the start of the first six generations. The percent of populations that went extinct and simple confidence intervals around each percent are provided.

3.2 | Growth rate through time

Density-dependent growth rates increased through generation four, then decreased again as population density increased (Figure 3a, Table S1). Density-independent growth rates were below or close to the replacement rate (growth rate of 1) in generations 1-3 (Figure 3b; confidence intervals overlap with dashed line). This changed in generation four, when the 0-migrant treatment had a mean density-independent growth rate much larger than the replacement rate, illustrating rapid adaptation within those populations (Figure 3). In generation four, density-independent growth rate in the 0-migrant populations was on average 0.808 (95% CI [0.207, 1.453]) higher than the 1-migrant populations and 0.969 (0.478, 1.573) higher than the 5-migrant populations. By generation five, all treatments gained and then maintained a mean density-independent growth rate greater than 1, showing adaptation to the environment. The comparison between the density-independent and density-dependent estimates of growth rates suggest that decrease in the latter by generation seven was likely due to the effects of negative density dependence, which increased as populations grew.

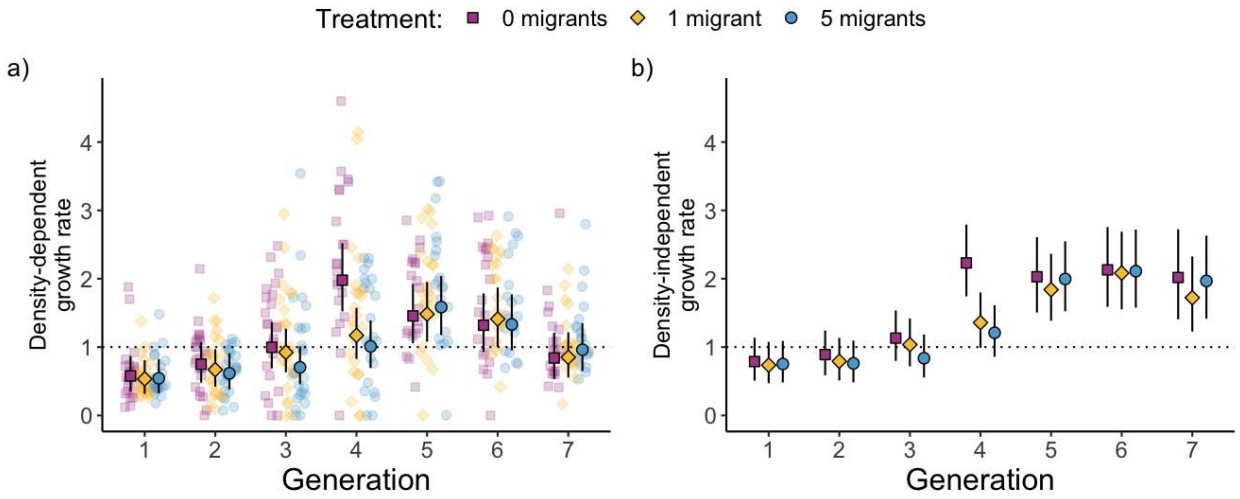

Figure 3. Density-dependent growth rate (a) and density-independent growth rate (b) throughout the rescue experiment. Each point outlined in black is the predicted mean for each treatment (purple

352 square = 0 migrants, yellow diamond = 1 migrant per generation, and blue circle = 5 migrants per
353 generation). Error bars are bootstrapped 95% confidence intervals. Points without black outlines are raw
354 growth rate values, and each point represents one population. The dotted line represents a growth rate
355 of 1, the replacement rate. Two estimates for growth rate are presented: a) the density-dependent
356 estimates are predictions from the linear mixed model for each generation and treatment combination,
357 used to incorporate the effects of negative density-dependence and provide estimates that describe the
358 raw data (shown), and b) the density-independent estimates (*i.e.,* using an initial population size of 0),
359 showing adaptation over time.
360
361 *Timing of evolutionary rescue*
362 Among the rescued populations only (black lines in Figure 2), the analysis of population
363 size with a GAM model revealed a shallow U-shaped curve (Figure 4). The estimated curves
364 from the best-fit model, which included treatment and the interaction of treatment and
365 generation as fixed effects and population identity as a random effect, are shown in Figure 4.
366 The interaction between immigration treatment and generation was significant ($\chi^2_3 > 330$, $p <$
367 0.0001), with the 0-migrant populations growing slightly faster than populations receiving
368 migrants (Figure 4). The 1-migrant and 5-migrant treatments maintained similar population
369 trajectories throughout the experiment.
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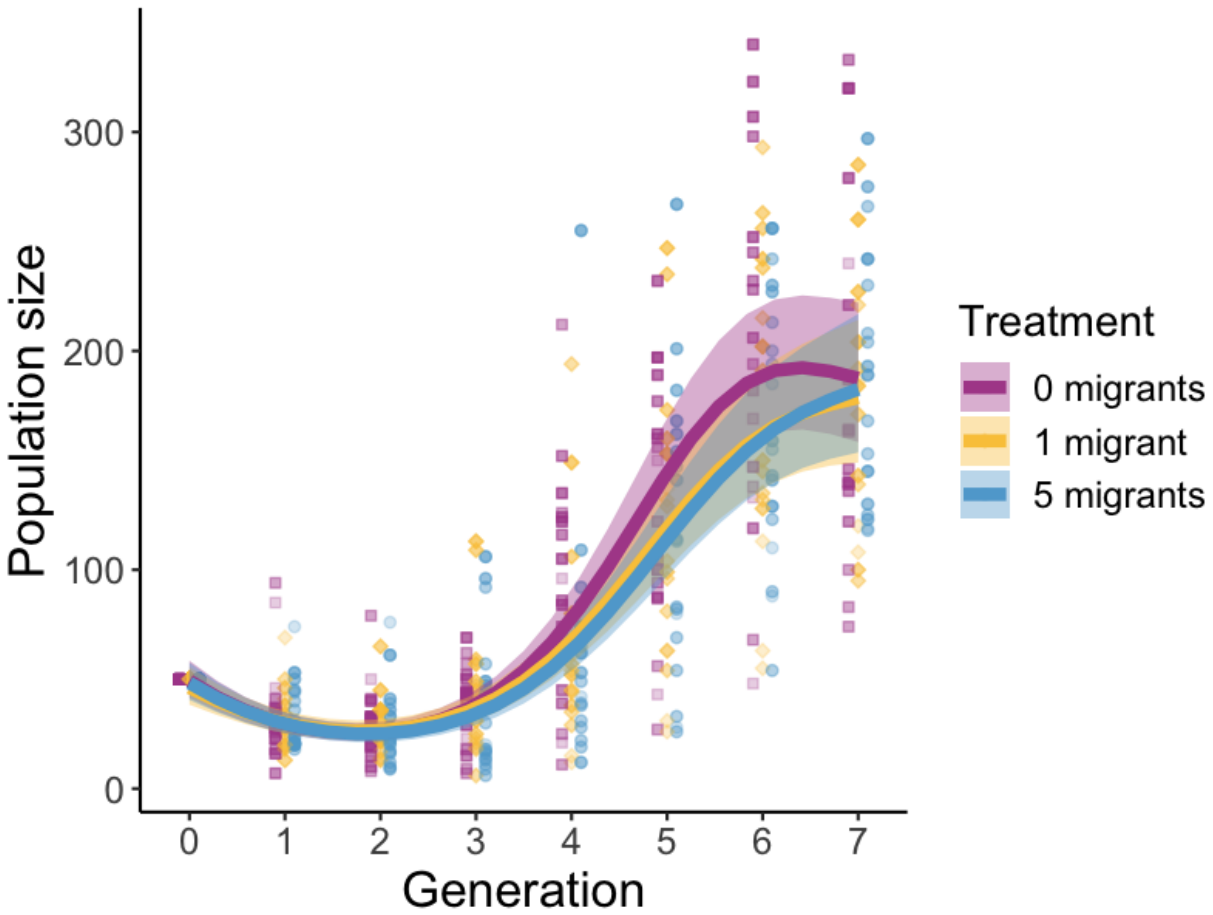


371

372 **Figure 4. Evolutionary rescue analysis.** Population size over time (in generations) of rescued

373 populations receiving 0 migrants (purple line, squares), 1 migrant (yellow line, diamonds), or 5

374 migrants (blue line, circles) per generation estimated by the best-fit GAM model. Each point

375 represents one population, which are staggered to reduce overlap. The lines are estimates of

376 mean population size for the three immigration treatments from the best-fit GAM model. The

377 shaded region around each line is the 95% confidence interval given by the GAM model.

378

379 *Adaptation and associated costs*

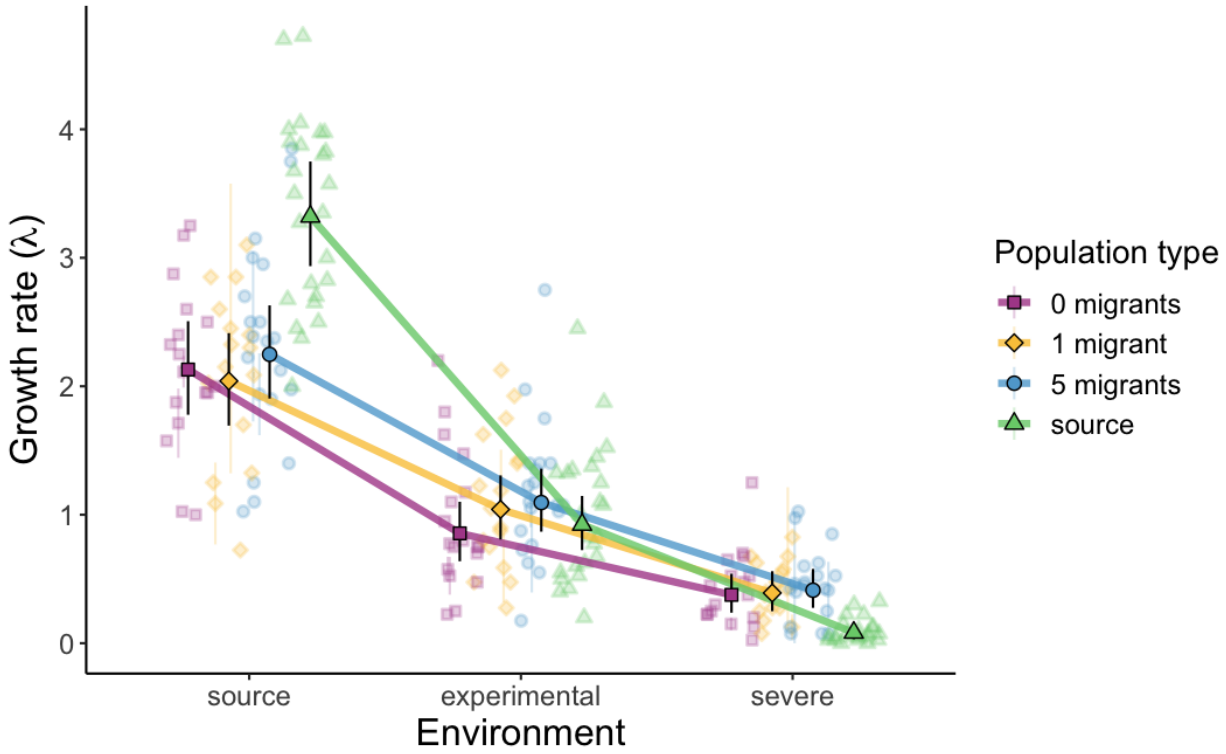
380 Growth rate in the reciprocal transplant experiment was influenced by an interaction

381 between environment and population type ($F_{6, 168.04} = 25.16$, $p < 0.0001$), in a pattern showing

that adaptation to the pesticide was linked to reduced fitness in the benign source environment (Figure 5). In the benign environment, the experimental populations had lower growth rates on average compared to the source populations (confidence intervals are negative and do not overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [-0.855, -1.628]; 95% $CI_{1\text{-migrant} - \text{source}}$ [-0.911, -1.748]; 95% $CI_{5\text{-migrant} - \text{source}}$ [-0.641, -1.482]), and a higher growth rate in the severe habitat (confidence intervals are positive and do not overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [0.442, 0.171]; 95% $CI_{1\text{-migrant} - \text{source}}$ [0.424, 0.210]; 95% $CI_{5\text{-migrant} - \text{source}}$ [0.464, 0.216]). Additionally, the 5-migrant populations performed marginally better than the 0-migrant populations in the experimental environment (95% $CI_{0\text{-migrant} - 5\text{-migrant}}$ [-0.0675, 0.572]).

Unexpectedly, the experimental and source populations performed similarly in the challenging experimental environment (Figure 5, all confidence intervals of the differences overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [-0.404, 0.209]; 95% $CI_{1\text{-migrant} - \text{source}}$ [-0.220, 0.403]; 95% $CI_{5\text{-migrant} - \text{source}}$ [-0.153, 0.452]). The experimental populations did not perform as well as expected based on their growth rates at the end of the rescue experiment (Figure 3). Our expectation for growth rates were based on predictions with N_{t-1} = 40 (the size used in the reciprocal transplant) from the linear mixed model created for the growth rate data in the rescue experiment. Those predicted values were 1.73, 95% CI [1.19, 2.34], 1.46 [1.02, 1.98], and 1.68 [1.21, 2.23] for the 0-migrant, 1-migrant, and 5-migrant treatments, respectively. The average growth rates we observed in the reciprocal transplant experiment in the experimental environment were lower than these estimates, with the means 0.86 [0.64, 1.10], 1.04 [0.81, 1.31], 1.09 [0.87, 1.36] that fall at the edge or outside the predicted values and CIs, and confidence intervals that overlap only slightly.

404 Furthermore, source populations did not perform as poorly as expected. We expected
405 them to perform similarly to the first generation of the experimental populations in the rescue
406 experiment prior to adaptation. The prediction given N_{t-1} = 40 was 0.62 [0.38, 0.92] for the 0-
407 migrant populations at the start of the rescue experiment, but the mean growth rate of source
408 populations was higher at 0.92 [0.72, 1.15]. The CIs overlap partially, but the overlap does not
409 include the mean, confirming that the source growth rates were unexpectedly high.



410

411 **Figure 5. Reciprocal transplant experiment results.** Growth rate was measured in three
412 environments: the source environment (standard dry media), the challenging experimental
413 environment, and a severe environment containing a higher concentration of insecticide. Each
414 point outlined in black is the model-estimated mean for each population type (purple squares =
415 0-migrant populations, yellow diamonds = 1-migrant populations, blue circles = 5-migrant
416 populations, and green triangles = source populations). Black error bars are bootstrapped 95%

417 confidence intervals; for the source populations in the severe environment (green), the black
418 error bars are not visible because they are too small to be seen. Thick lines connecting these
419 points emphasize the treatment-environment interaction. Each point without the black outline
420 represents a single population, with the light error bars representing raw 95% confidence
421 intervals across replicates; points without error bars have no replicates. Points are staggered
422 and jittered to reduce overlap.
423
424

425 4 | Discussion

426 Our study evaluated the effects of different immigration rates on adaptation to a new,
427 challenging environment using experimental populations of *Tribolium castaneum*. We
428 evaluated differences between initially declining populations receiving two immigration rates
429 (one or five migrants per generation) with isolated populations that received no immigration.
430 The migrants were not adapted to the challenging environment, allowing us to examine the
431 degree to which immigration can limit adaptation (Savolainen *et al.*, 2013). While the
432 experimental populations declined in size initially, leading some populations to go extinct,
433 around 80% of populations were able to persist for the duration of the seven-generation
434 experiment. Most of these populations had growth rates above the replacement rate and had
435 large population sizes, characteristic of evolutionary rescue. We also found that extinction rate
436 was similar regardless of immigration. However, growth rates increased one generation sooner
437 in the isolated populations compared to those with immigration. This delay was brief, as the
438 following generation, growth rates were comparable across the different immigration

439 treatments. We also tested for adaptation using a reciprocal transplant experiment. While
440 some of the results from that experiment were unexpected, overall it provided evidence that
441 our experimental populations were adapted to the insecticide that created the challenging
442 environment. There was also a cost to that adaptation, evident from the experimental
443 populations having lower growth rates than the sources in the benign environment.
444
445 *Observed delay in the increase in growth rate: possible mechanisms*
446 Populations without immigration gained a much higher density-independent growth
447 rate (more than doubling on average) compared to the populations with immigration (near or
448 just above the replacement rate) in the fourth experimental generation. The following
449 generation, all populations grew at a similar rate regardless of immigration. The delay we
450 observed may have been due to *i)* differences in density dependence, *ii)* phenotypic plasticity,
451 or *iii)* outcrossing with naive migrants slowing adaptation to the challenging environment.
452 First, theory shows that reduced negative density dependence in a degraded habitat
453 may enhance rescue (Uecker *et al.*, 2014; Czuppon *et al.*, 2021). In our system, population
454 densities were low in the experimental populations in the initial generations, and thus negative
455 density-dependent processes were likely relatively weak. The added immigrants increased
456 density slightly relative to the treatment without migrants, and this could have contributed to
457 lower growth rates. However, if this were the case, the populations receiving five migrants each
458 generation should have experienced further reductions in growth than the populations
459 receiving one per generation, as their density was increasing by a greater amount (31% increase
460 for the 5-migrant populations on average compared to a 7% increase for the 1-migrant

461 populations). Thus, density dependence is an unlikely explanation for the one-generation delay
462 in increased growth rates.

463 Second, growth rate in the challenging environment could be influenced by phenotypic
464 plasticity, which could have led to the lag in increased growth rates. Recently, it has been
465 shown that plasticity in insects can be mediated by associated microbes (Kolodny &
466 Schulenburg, 2020). Variation in the microbial communities in host insects is high (Lange *et al.,*
467 2023), and the microbial community can evolve, which can facilitate persistence in new and
468 challenging environments (Kolodny & Schulenburg, 2020). Microbes, therefore, can serve as an
469 important source of adaptive phenotypic plasticity (Kolodny & Schulenburg, 2020; Ghalambor
470 *et al.,* 2007). Insect-associated microbes can be transmitted from parent to offspring (Lange *et*
471 *al.,* 2023), indicating that plasticity could be multi-generational. If the microbial community
472 influences fitness in the presence of deltamethrin, it is possible that plasticity and adaptation in
473 the microbiome helped facilitate the observed increase in growth rate during the rescue
474 experiment. The delay in increase for populations with immigration could be due to the
475 microbiome from the benign environment being continually re-introduced by the migrants.

476 Third, migration is well known as a constraint to adaptation (Slatkin, 1987). For example,
477 theory has shown that immigration between different habitat types can limit local adaptation
478 through genetic swamping of adaptive alleles (Lenormand, 2002; Bolnick & Nosil, 2007), and
479 reduce the probability (Schiffers *et al.*, 2013) or speed (Holt & Barfield, 2015) of evolutionary
480 rescue because of maladaptation in the new environment. It seems likely that migration of
481 naive individuals slowed adaptation to the novel environment, which may have been enhanced
482 by the mating behavior of *T. castaneum*. In this species, males reared in high-quality

483 environments have increased mating rate and insemination success compared to males reared
484 in poor environments (Lewis *et al.*, 2012). Thus, male migrants from the benign source
485 environment may have successfully mated with multiple females in the experimental patches,
486 enhancing the rate of gene flow of maladapted alleles. Female migrants would have also likely
487 spread maladapted alleles, as a single female *T. castaneum* can lay over 10 eggs per day (Pray
488 et al., 1949). Thus, regardless of sex, outcrossing with the naive migrants likely contributed to
489 the brief delay in growth rate increase. Given the strength of selection pressure, however,
490 adaptation likely enabled growth rates to increase in the following generation, which we
491 observed on average across all treatments.

492 We suggest that the increases in population size and growth rate, likely through
493 adaptation and possibly aided by adaptive plasticity, can be described as evolutionary rescue.
494 We further evaluate the effects of adaptation *vs.* plasticity when we discuss the reciprocal
495 transplant experiment below. The finding that rescue can occur with intermediate levels of
496 immigration of not adapted individuals is supported by several modeling studies that assess
497 evolutionary rescue in heterogenous, structured systems (Gomulkiewicz *et al.*, 1999; Uecker *et*
498 *al.*, 2014; Tomasini & Peischl, 2020; Czuppon *et al.*, 2021). This result has also been shown in a
499 case study of Trinidadian guppies, where rescue was able to occur with gene flow from a
500 maladapted source population (Fitzpatrick *et al.,* 2014, 2020).

501

502 *Observed delay in rescue: implications*

503 Our finding of only a brief (one generation) delay of adaptation has practical
504 implications. Such delayed adaptation could be detrimental to populations at imminent risk of

extinction due to small size (Gomulkiewicz & Holt, 1995; Melbourne & Hastings, 2008). However, for populations not at immediate risk of extinction, such a delay may be inconsequential, and worth the potential increase in genetic diversity that occurs through immigration. Such increased diversity may prevent inbreeding depression from developing, and improve the ability of the population to adapt to future environmental changes (Frankham *et al.,* 2019).

Thus, these results suggest that naive migrants can be suitable to use in management efforts. Furthermore, they are often more feasible to obtain, either from captive rearing programs (Crone *et al.,* 2007) or from large source populations living in a different habitat (*e.g.,* different host plants in Bisschop *et al.,* 2019) or geographic region (*e.g.,* isolated guppy populations in Fitzpatrick *et al.,* 2020), when habitat matching is not possible.

Additionally, our findings are consistent with refuge strategies to delay evolution of pest resistance to toxins, such as in crops genetically modified to express *Bacillus thuringiensis* (*Bt*). The immigration of susceptible individuals from sections of fields planted in non-*Bt* crops, or growing crops in areas with plentiful alternative host plants for the pest has proven to be highly effective (Carrière *et al.*, 2012). In those situations, particularly with 50% or more of a pest population being susceptible, resistance can be successfully managed for multiple generations (Tabashnik *et al.*, 2008). Given our much smaller proportion of susceptible individuals, it makes sense that the delay of the evolution of resistance was brief.

Reciprocal transplant experiment: interpretation of unexpected results

The two most straightforward takeaways from the reciprocal transplant experiment are *i)* the experimental populations had higher growth rates than the source populations in the severe environment, suggesting they had adapted to the insecticide used to create the challenging environment in the rescue experiment and *ii)* the experimental populations had lower growth rates in the benign environment than the experimental populations, suggesting that there was a cost associated with adaptation to the insecticide. However, the source populations had higher than expected growth rates in the experimental environment, and the experimental populations had lower than expected growth rates in the experimental environment inconsistent with the hypothesis of adaptation. We next discuss *i)* how experimental challenges might have affected the source populations, *ii)* how the common environment generation could have reduced growth rates by eliminating effects of plasticity, and *iii)* how multiple pathways of adaptation in the experimental populations could explain their performance in the reciprocal transplant experiment.

First, the high performance of the source populations in the experimental environment could be due to challenges in implementing the experimental design. For instance, accidental contamination of the source population with deltamethrin, or accidental gene flow from the experimental to the source populations could have occurred during the rescue experiment. We took great care to avoid both forms of contamination, but if contamination nevertheless occurred, it could have allowed the source populations to adapt to low concentrations of the insecticide. The source populations were not adapted to high concentrations of the pesticide, as evidenced by their performance in the severe environment.

549 A second consideration is that the common environment generation could have masked
550 differences in growth rates, if they were initially caused by plasticity. As described above,
551 microbiome-mediated phenotypic plasticity could have contributed to the increase in growth
552 rate in the rescue experiment (Lange *et al.,* 2023). If microbiome-mediated plasticity drove
553 performance, the generation in a common environment prior to the reciprocal transplant
554 experiment would have alleviated selection pressure and reduced the ability of the
555 experimental populations to tolerate the insecticide. This could help explain the similar
556 performance of the experimental and source populations in the experimental environment.
557 However, there does not appear to be strong evidence for the masking of phenotypic plasticity
558 in the severe or benign environments given that the experimental populations had a much
559 higher growth rate than the sources in the severe and a lower growth rate in the benign. We
560 suggest that those differences can be explained by our last point, adaptation, which is discussed
561 below.

562 Finally, adaptation to deltamethrin likely explains the relative performance of the
563 populations in the three environments. More specifically, it is possible that the experimental
564 populations adapted through more than one mechanism during the rescue experiment. First,
565 adaptation to detoxify the insecticide compound likely occurred, as rapid evolutionary
566 responses to deltamethrin is well documented in pest populations of *T. castaneum* (Collins,
567 1998; Stuart *et al.*, 1998) through the increased expression of cytochrome P450 enzymes (Kalsi
568 & Palli, 2015). In other insect species, this mechanism of resistance to deltamethrin has a
569 confirmed cost (Tchouakui *et al.*, 2020), and such a cost is consistent with the reduced
570 performance of experimental populations in the benign environment. The second way that

571 populations could have adapted is by increasing the rate of egg cannibalism to supplement
572 their diets, as cannibalism propensity is a genetically based trait that can evolve (Stevens, 1989)
573 that has been shown to enhance fitness in challenging environments (Via, 1999; Pointer *et al.,*
574 2021). These potential pathways, evolution of increased detoxification and of increased
575 cannibalism, are not mutually exclusive, and both may have facilitated increased population
576 growth in the experimental environment during the rescue experiment. Then, in the reciprocal
577 transplant experiment, the populations were initiated at lower density (40 parents) in the three
578 environments. In this lower density environment, the number of eggs available for cannibalizing
579 was reduced compared to what was available in the latter half of the rescue experiment (in
580 generations 4-7, $N_{t-1} > 150$ on average). Thus, the decreased density reduced the eggs available
581 for cannibalism, limiting one of the adaptive pathways and reducing overall growth rates of the
582 experimental populations in the experimental environment. In the severe environment, growth
583 rates of the experimental populations were low but still greater than the source populations,
584 which can be explained primarily by their evolved ability to detoxify the pesticide.
585
586 *Considerations for future experiments*
587 The bottleneck events that occurred during the rescue experiment, some as small as 10
588 individuals, would likely have longer-term consequences (Frankham *et al.*, 1999; Olazcuaga *et*
589 *al.*, 2023). Census population size in a wide range of natural populations is almost always larger
590 than the effective population size (the median N_e/N_c ratio across plant and animal taxa is
591 approximately 0.1; for *T. castaneum,* it is estimated to be between 0.5-0.9) and bottlenecks of
592 $N_e < 50$ can result in reductions in genetic variation and increases in inbreeding (Wade, 1980;

593 Jamieson & Allendorf, 2012). For this reason, we suggest that future work examine the effects
594 of demographic bottlenecks of this severity by propagating experiments like this for longer (*i.e.,*
595 more than 8 generations). Longer-term experiments would be able to test the hypothesis that
596 the benefits of immigration become more apparent over longer time scales. Such experiments
597 would help shed light on the longer-term impacts of immigration for small and isolated
598 populations, an important question when considering whether to implement translocations in
599 declining natural populations (Frankham, 2015, 2016). Genomic methods would also provide
600 important insights for monitoring the genetic contributions of migrants, the genetic
601 differentiation between the experimental and source populations, and for confirming the genes
602 contributing to adaptation (Whiteley *et al.*, 2015). Genomics would also allow us to more
603 clearly differentiate between adaptation and plasticity (Koch & Guillaume, 2020).
604
605 *Conclusions*
606 This study explored the effects of immigration of naive individuals on large, outbred,
607 and recently diverged populations following population decline due to sudden environmental
608 degradation. Following such an event, the ideal solution would be to restore the habitat, but
609 large-scale restoration efforts are often very slow or impractical in the short-term (Frankham *et*
610 *al.,* 2019). Here, we provide support for using immigration as a tool to sustain populations for
611 multiple generations until restoration can occur (Whiteley *et al.*, 2015; Frankham *et al.*, 2019).
612 To our knowledge, we are the first to experimentally test the effects of the recommended
613 immigration rates of one compared to five migrants per generation on adaptation in a
614 challenging environment. We found that though immigration slowed adaptation in the short-

term, extant populations with and without immigration were on average equally well adapted to severe conditions after seven generations. Thus, we confirm the findings of previous theoretical work (Tomasini & Peischl, 2020; Czuppon *et al.*, 2021) by demonstrating experimentally that evolutionary rescue can occur both with and without immigration following severe environmental change.

Data Availability Statement

The data and R scripts for our analyses are available at: https://anonymous.4open.science/r/tribolium-rescue-DB19/README.md. They will be archived on Dryad upon acceptance of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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